

FLAVONOID GLYCOSIDES OF *LEUCOSPERMUM* R.BR.

C. W. GLENNIE*

(Department of Chemistry, University of the Witwatersrand, Johannesburg)

ABSTRACT

Flavonoids were surveyed (by paper chromatography) in leaves of 29 *Leucospermum* species. All samples examined contained the 3-O-galactosides and 3-O-rutinosides of quercetin and isorhamnetin while the 3-O-glucosides and 3-O-arabinosides of these aglycones were more restricted in their distribution. Myricetin 3-O-rutinoside was very restricted, occurring in only 5 species. The taxonomic significance of this distribution pattern as well as the phylogenetic implications are discussed. No cyanogenic compounds could be detected and arbutin was also absent. The phenolic lactone, leucodrin, was found in 9 of the species surveyed.

UITTREKSEL

FLAVONOIDGLIKOSIEDE VAN *LEUCOSPERMUM* R.BR

Die voorkoms van flavonoïede is deur papier-chromatografie bepaal op blare van 29 *Leucospermum* spesies. Al die monsters wat ondersoek is bevat die 3-O-galaktosiede en 3-O-rutinosiede van kwersetien en isorhamnetien terwyl die 3-O-glukosiede en 3-O-arabinosiede van hierdie aglikone meer beperk is in hulle distribusie. Mirisetien-3-O-rutinosied is baie meer beperk en het in 5 spesies voorgekom. Die taksonomiese betekenis van hierdie distribusiepatroon asook die filogenetiese implikasies word bespreek. Geen sianogeniese stowwe kon ontdek word nie en arbutien is ook afwesig. Die fenoliese laktoon, leucodrin, is teenwoordig in 9 van die spesies wat ondersoek is.

INTRODUCTION

The genus *Leucospermum* currently comprises 47 species arranged in 9 sections (Rourke, 1972). This genus is found in the sub-family Proteoideae of the Proteaceae and it is this sub-family which contains most of the species indigenous to South Africa; the other sub-family, Grevilleoideae, comprises mainly Australian species. The present survey covers 29 species representing all 9 sections described by Rourke. The principal reason for undertaking this study was to improve our knowledge of the phenolics, in particular the flavonoids, found in *Leucospermum*, and also to see if they could aid in an understanding of the classification and phylogeny of this genus.

Some *Leucospermum* spp. have been examined previously for their phenolic content. Leucodelphinidin, leucocyanidin, myricetin, quercetin, kaempferol and caffeic acid have been reported in 12 species (van Oudtshoorn, 1963; Hegnauer, 1969; Elsworth and Martin, 1971): van Oudtshoorn also reported the presence of arbutin and hydroquinone in all of the 5 species he examined.

That flavonoids are now used regularly as chemical markers in plant taxonomy

*Present address: Council for Scientific and Industrial Research, Pretoria.

Accepted for publication 28th August, 1978.

has been ably discussed by Harborne (1967, 1975). The studies mentioned above involving phenolics of the Proteaceae have used the presence or absence of leucoanthocyanins and the occurrence of the three vicinal hydroxy groups as found in myricetin as important markers. Certain flavonoid characters have been related to morphological advancement and hydroxylation patterns are important characters which can aid in establishing phylogenetic sequences. Elsworth and Martin divided their plants into four broad groups of different evolutionary advancement depending on the presence or absence of certain flavonoid patterns.

Since all previous studies were done on acid-hydrolysed material, only the flavonoid aglycones were isolated and identified. In view of this, the present study deals with aqueous methanolic extracts which contain the flavonoid glycosides. Also, many *Leucospermum* spp. not examined before have been included in this report. Along with the flavonoids the occurrence of the novel phenolic lactone, leucodrin, is tabulated and all samples were surveyed for the presence of arbutin and cyanogenic compounds.

Leucodrin has been known since 1886 but its structure was only established in recent years (Perold and Pachler, 1966). It has been isolated from various *Leucadendron* spp. and is characteristic of this genus having been found in all species examined. Conocarpin, which is a diastereoisomer of leucodrin (Perold, Hodgkinson and Howard, 1972b), is characteristic of the *Leucospermum* genus. It was first isolated and identified from *Leucospermum conocarpodendron* (Kruger and Perold, 1970). Related compounds, reflexin and conocarpic acid as well as conocarpin itself have been isolated from *L. reflexum* (Perold, Hodgkinson and Howard, 1972a).

METHODS

The plant material was collected from the Botanical Research Institute, Pretoria and the National Botanic Gardens, Kirstenbosch. Voucher specimens are deposited in the herbaria of these two institutions. The material was either air-dried and milled before extraction with 80% methanol or the fresh material was ground in a Waring Blendor directly with 80% methanol. The flavonoid glycosides were extracted, separated, purified and identified by standard procedures (Harborne, 1967). R_f values and uv spectral data for the flavonoids described are found in Table 1.

For comparison purposes two-dimensional paper chromatograms were run on Whatman No. 1 paper using PhOH (phenol:water—500:125) and 15% HOAc. To improve viewing under uv light the dried chromatograms were dipped in 3% $AlCl_3$ in methanol. This changed the flavonoids from dark absorbing spots to bright yellow spots which were more easily and reliably scored. All plant extracts were examined for leucodrin by paper chromatography on paper pretreated with 10% glycerol in methanol and run in BuOH:Tol (n-butanol:toluene-1:1 and saturated with H_2O). After air-drying, the chromatograms were sprayed with Pauly's

reagent (0.5% solution of diazotised sulphanilic acid in 10% aqueous Na_2CO_3). The extracts were also examined for hydrogen cyanide producing compounds and arbutin according to methods previously described (Glennie and Davidson, 1978).

TABLE 1.
R_f and spectral characteristics of the flavonoid glycosides of *Leucospermum*.

Glycoside	BAW	R _f (x 100) in		Phenol*
		15% HOAc	H ₂ O	
Quercetin-3-O-rutinoside	40	53	28	46
Quercetin-3-O-glucoside	75	45	10	56
Quercetin-3-O-galactoside	59	45	12	48
Quercetin-3-O-arabinoside	53	39	12	53
Isorhamnetin-3-O-rutinoside	43	61	37	79
Isorhamnetin-3-O-glucoside	65	37	05	73
Isorhamnetin-3-O-galactoside	57	45	15	75
Isorhamnetin-3-O-arabinoside	69	24	04	80
Myricetin-3-O-rutinoside	54	50	10	35

Glycoside	λ max (nm) in 80% MeOH		NaOAc	$\Delta\lambda$ max (nm) in AlCl ₃ NaOMe		H ₃ BO ₃
	Band I	Band II	Band I	Band II	Band II	Band II
Quercetin 3-O-glycoside	256 (265) [†]	358	18	62	42	22
Isorhamnetin 3-O-glycoside	257 (265)	360	18	42	54	0
Myricetin 3-O-rutinoside	264	356	6	67	decomposes 20	

*BAW—organic layer of n-butanol:acetic acid:water (4:1:5); 15%HOAc—15% acetic acid; Phenol—500 g phenol in 125 g water
[†]inflection

DISCUSSION

The distribution of the different flavonoid glycosides throughout the 9 sections of *Leucospermum* are shown in Table 2. All the plants listed have been screened for all the compounds listed and only positive results presented. Blanks in the table represent negative results.

In all cases the sugars are attached at the 3-O-position. The galactosides and rutinosides of quercetin and isorhamnetin appear to be distributed uniformly throughout the genus while other glycosides have a more restricted distribution. Arabinosides of isorhamnetin and quercetin show a limited occurrence as do the glucosides of these aglycones, i.e. sections 2, 4, 5 and 6 contain arabinosides and lack glucosides while sections 1 and 3 contain glucosides and lack arabinosides. The arabinosides are more widespread than the glucosides and it should be noted that they do not usually occur together except in sections 7, 8 and 9 where

quercetin glucoside occurs with both arabinosides. Myricetin rutinoside is restricted to a few species in only four sections.

Elsworth and Martin reported kaempferol present in all *Leucospermum* species they examined and van Oudtshoorn scored most of his *Leucospermums* for kaempferol. In this present study no kaempferol could be detected but rather isorhamnetin was isolated from all 29 species. Since it is rather difficult to distinguish between kaempferol and isorhamnetin in most chromatographic systems I would suggest that it was isorhamnetin rather than kaempferol that the above authors detected. In this study the isorhamnetin agreed with authentic material in R_f values in 4 different solvents and in uv spectral characteristics; mass spectral data also indicated isorhamnetin rather than kaempferol.

Like isorhamnetin, quercetin was found in every species examined, being most commonly found as the rutinoside and galactoside. Myricetin is more restricted in its distribution and was found in only 5 out of 29 species surveyed. The *Leucospermum* spp. listed in Table 2 do not contain cyanogenic compounds nor do they contain the glucoside of hydroquinone, arbutin. *Protea* spp. are rich in arbutin (Glennie and Davidson, in press; van Oudtshoorn, 1963) but although van Oudtshoorn reported arbutin present in all *Leucospermum* spp. he surveyed, none could be detected in this study.

Two unusual phenolic lactones occur in 9 of the species surveyed; these lactones are leucodrin (Perold and Pachler, 1966) and its companion compound, leudrin (Hight, Perold and Sokoloski, 1976). Leucodrin is present in all species of *Leucadendron* tested for its presence while leudrin has a slightly more restricted distribution. Since these unusual compounds are found in both *Leucadendron* and *Leucospermum* spp. it could be suggested that both these genera are related and perhaps even have ancestry in common.

It should be noted that most species containing leucodrin and leudrin occur in the last 4 sections in Table 2. *Leucospermum* spp. which do not contain leucodrin contain its diastereoisomer, conocarpin. Since both of these compounds are rather complex molecules and also are isomers of each other, it would appear that their biosynthetic pathways did not evolve independently. It is more probable that one is a replacement character of the other, and the presumed more advanced species of this genus could have gained the ability to biosynthesize leucodrin as they lost their ability to produce its isomer, conocarpin.

On morphological and phylogeographic grounds, Rourke regards the species in Section 1 (*Crassicaudex*) as the most primitive and those in Sections 6, 7, 8 and 9 (*Leucospermum*, *Diastelloidea*, *Xericola* and *Crinitae*) as the most advanced. He suggests that it is from these advanced sections (6–9) that the possible ancestors of *Leucadendron* could have been derived. The chemical data reported in Table 2 would support this in that it is the last four sections which contain the species biosynthesizing leucodrin which is typical of *Leucadendrons* and not conocarpin which is characteristic of the presumed more primitive *Leucospermums*.

In placing their plants into four different groups, Elsworth and Martin found both *Leucospermum* and *Leucadendron* in Groups I and II (their most primitive groups) and *Leucadendron* only in Group III (a more advanced group) suggesting that at least some species of *Leucadendron* are more advanced than *Leucospermum*. The 5 species of *Leucospermum* from Rourke's Sections 6–9 that they studied were placed in Group I while the remaining 10 species from Rourke's more primitive sections were found in Group I (4 species) and Group II (6 species). This grouping was chosen mainly on the presence or absence of leucoanthocyanins and myricetin with its three vicinal hydroxy groups. In the present study most plants found to contain myricetin occurred in Rourke's more primitive sections. Work is continuing in an attempt to identify the flavonoid content as well as unusual phenolics in both *Leucospermum* and *Leucadendron*. This could possibly help clarify the position of these two genera and aid in understanding their phylogeny.

Suggesting that *Leucospermum* and *Leucadendron* are closely related and possibly had a common ancestry is contrary to the phylogenetic relationships inferred by Johnson and Briggs (1975). They suggested that *Leucadendron* and *Aulax* are derived from Aulacinae and *Leucospermum* and other genera with n:12 come from Proteinae. When the distribution of the flavonoids in *Leucadendron* is established it will aid in determining the relationships between the genera within the Proteoideae.

The Tumiditubus section consists of 8 species, 6 of which are included in this survey. At one end of the range is the *L. praecox*/*L. fulgens* group which are erect, rounded shrubs and from this the form gradually moves to the *L. spathulatum*/*L. profugum* group which are sprawling shrubs. There is also a significant change in flavonoid content as the erect shrubs contain only isorhamnetin and quercetin glycosides while the sprawling shrubs at the other end of the scale do not contain quercetin glucoside but rather myricetin rutinoside and leucodrin with a gradual transition between the two extremes.

It would be worthwhile to extend this study to as many taxa as possible within the Proteoideae for several reasons. The flavonoid patterns could be of value in determining the relationships between the various taxa within this sub-family and also in establishing their phylogeny. Since the Proteaceae appears to be rich in unusual phenolic compounds it would certainly be worthwhile to survey as many of its members as possible for these novel compounds. Lastly, it would be interesting to examine Grevilleoideae in the same manner and see how the two sub-families compare with each other.

ACKNOWLEDGEMENTS

Professor G. W. Perold (Department of Chemistry, University of the Witwatersrand) is thanked for his encouragement and for his support of this work. The Director, Botanical Research Institute, Pretoria, and Dr J. P. Rourke, National

Botanic Gardens, Kirstenbosch, are thanked for providing plant material and the latter is thanked for helpful discussions.

TABLE 2.

Distribution of Phenol Glycosides in <i>Leucospermum</i> Species											
Species	1	2	3	4	Compound						
	5	6	7	8	9	10	11				
Sect. 1. CRASSICAUDEX Rourke											
<i>L. saxosum</i> S. Moore	+	+	+	+			+	+			
<i>L. cuneiforme</i> (Burm. f.) Rourke	+	+	+	+			+	+			
<i>L. innovans</i> Rourke	+	+	+	+			+	+			
Sect. 2. CONOCARPODENDRON Buek ex Endl											
<i>L. conocarpodendron</i> (L.) Buek	+	+	+	+	+	+					
<i>L. glabrum</i> Phillips	+	+	+	+	+	+					
<i>L. pluridens</i> Rourke	+	+	+	+	+	+					
Sect. 3. TUMIDITUBUS Rourke											
<i>L. praecox</i> Rourke	+		+	+			+	+			
<i>L. fulgens</i> Rourke	+		+	+			+	+			
<i>L. muirii</i> Phillips	+		+	+			+	+			
<i>L. erubescens</i> Rourke	+		+	+				+			
<i>L. utriculosum</i> Rourke		+	+	+			+			+	+
<i>L. spathulatum</i> R. Br.	+		+	+				+	+	+	+
Sect. 4. BREVIFILAMENTUM Rourke											
<i>L. vestitum</i> (Lam) Rourke	+	+	+	+	+	+					
<i>L. tottum</i> (L.) R. Br.	+	+	+	+	+	+					
<i>L. lineare</i> R. Br.	+	+	+	+	+	+					
<i>L. cordifolium</i> (Salisb. ex Knight)											
Fourcade	+	+	+	+	+	+			+		
<i>L. cordatum</i> Phillips	+	+	+	+	+	+					
Sect. 5. CARDINISTYLUS Rourke											
<i>L. formosum</i> (Andr.) Sweet	+	+	+	+	+	+					
<i>L. catherinae</i> Compton	+	+	+	+	+	+					
<i>L. queinzii</i> Meisn.	+	+	+	+	+	+			+		
<i>L. reflexum</i> Buek ex Meisn.	+	+	+	+	+	+			+		
Sect. 6. LEUCOSPERMUM											
<i>L. tomentosum</i> (Thunb) R. Br.	+	+	+	+	+	+			+	+	+
Sect. 7. DIASTELLOIDEA Phillips											
<i>L. calligerum</i> (Salisb. ex Knight)											
Rourke	+	+	+	+	+	+	+			+	+
<i>L. royenifolium</i> (Salisb. ex Knight)											
Stapf	+	+	+	+	+	+	+			+	+
<i>L. truncatulum</i> (Salisb. ex Knight)											
Rourke	+	+	+	+	+	+				+	+
<i>L. bolusii</i> Gandoger	+	+	+	+	+	+	+			+	+
Sect. 8. XERICOLA Rourke											
<i>L. alpinum</i> ssp. <i>amoenum</i> Rourke	+	+	+	+	+		+			+	+
Sect. 9. CRINITAE Phillips											
<i>L. oleifolium</i> (Berg) R. Br.	+	+	+	+	+	+	+				
<i>L. mundii</i> Meisn.	+	+	+	+	+	+	+			+	+

KEY

1. Quercetin 3-O-rutinoside;
2. Isorhamnetin 3-O-rutinoside;
3. Quercetin 3-O-galactoside;
4. Isorhamnetin 3-O-galactoside;
5. Quercetin 3-O-arabinoside;
6. Isorhamnetin 3-O-arabinoside;
7. Quercetin 3-O-glucoside;
8. Isorhamnetin 3-O-glucoside;
9. Myricetin 3-O-rutinoside;
10. Leucodrin;
11. Leudrin.

REFERENCES

- ELSWORTH, J. F. and MARTIN, K. R., 1971. Flavonoids of the Proteaceae. Part 1. A Chemical Contribution to Studies on the Evolutionary Relationships in the S. African Proteoideae. *Jl S. Afr. Bot.* **37**: 199–212.
- GLENNIE, C. W. and DAVIDSON, L. E., in press. Flavonoids of the Summer Rainfall Proteas and their Chemotaxonomic Significance. *Jl S. Afr. Bot.*
- HARBORNE, J. B., 1967. *Comparative Biochemistry of the Flavonoids*. London and New York: Academic Press.
- HARBORNE, J. B., 1975. The Biochemical Systematics of Flavonoids. In: Harborne, J. B., Mabry, T. J. and Mabry, Helga, (eds.) *The Flavonoids*. London: Chapman and Hall.
- HEGNAUER, R., 1969. *Chemotaxonomie der Pflanzen*. Band 5, p. 407. Basel and Stuttgart: Birkhauser Verlag.
- HIGHET, R. J., PEROLD, G. W. and SOKOLSKI, L. A., 1976. Characterization of Spiro-bis-lactonic Phenolic Metabolites of Proteaceae by ¹³C Nuclear Magnetic Resonance. *J. org. Chem.* **41**: 3860–3862.
- JOHNSON, L. A. S. and BRIGGS, B. G., 1975. On the Proteaceae—the Evolution and Classification of a Southern Family. *J. Linn. Soc., Bot.* **70**: 83–182.
- KRUGER, P. E. J. and PEROLD, G. W., 1970. Conocarpin, a Leucodrin-type Metabolite of *Leucospermum conocarpodendron* (L.) Buek *J. chem. Soc. (C)*: 2127–2133.
- PEROLD, G. W. Unpublished results.
- PEROLD, G. W., HODGINKINSON, A. J. and HOWARD, A. S., 1972a. Metabolites of Proteaceae. Part V. Reflexin and Conocarpic Acid from *Leucospermum reflexum* Buek ex Meisner, and the Phenol-Dienone. Rearrangement of Reflexin and Conocarpin. *J. chem. Soc. Perkin 1*: 1972, 2450–2457.
- PEROLD, G. W., HODGINKINSON, A. J. and HOWARD, A. S., 1972b. Metabolites of Proteaceae. Part VI. The Stereochemistry of Reflexin and Conocarpin. *J. chem. Soc. Perkin 1*: 1972, 2457–2460.
- PEROLD, G. W. and PACHLER, K. G. R., 1966. The Structure and Chemistry of Leucodrin. *J. chem. Soc. (C)*: 1918–1923.
- ROURKE, J. P. Private communication.
- ROURKE, J. P., 1972. Taxonomic Studies on *Leucospermum R. Br.* *Jl S. Afr. Bot. Suppl.* vol. **8**.
- VAN RHEEDE VAN OUDTSHOORN, M. C. B., 1963. Distribution of Phenolic Compounds in some South African Proteaceae. *Planta med.* **11**: 399–406.